specific ion channel inhibition, e.g. to allow selective antiarrhythmic drug development for the treatment of atrial fibrillation. Here, we sought to compare voltage-gated sodium currents (I_{NA}) in atrial and ventricular cardiomyocytes in mice, as Na^+ channel subunits have been found to differ between atria and ventricles in rat and man.

Aim The aim of this study is to examine whether biophysical properties of I_{NA} are altered in mouse atrial cardiomyocytes compared to left ventricular cardiomyocytes.

Methods Na+ channel currents were measured using whole-cell voltage clamp in left atrial and left ventricular cardiomyocytes. Expression of Nav1.5 proteins and their regulatory ?-subunits was measured by western blotting in left atrial, right atrial and left ventricular tissue of wild-type 129/sv mice (15-20 weeks). Protein levels were normalised against calnexin.

Results Mean peak INA was significantly increased in left atrial myocytes compared to left ventricular (LA=−28.63±1.856pA/pF; n=15/4 cells/mice; LV =-19.83±4.186pA/pF; n=5/2 cells/mice; *p<0.05) and V50 for INA inactivation was significantly more negative in left atrial compared to left ventricular myocytes (LA=−92.4±1.877mV; n=16/4 cells/mice; LV=−81.77 ±2.413mV; n=5/2 cells/mice; *p<0.01). No difference in Nav1.5 expression was detected between chambers, however, expression of ÃÂ²2 and ÃÂ²4 subunits was significantly reduced in atrial tissue compared to left ventricular (LA=0.189 ±0.02014; RA=0.3023±0.0333; LV=0.736±0.0718; *p<0.01; n=4) and (LA=0.00145±0.00033; RA=0.00204±0.00102; LV=0.0214±0.000613; *p<0.01; n=4) respectively.

Conclusion Mouse atrial cardiomyocytes display increased INA compared to cardiomyocytes isolated from the ventricles. Alterations in biophysical properties of INA in mouse atrial myocytes may be attributable to reduced expression of the Nav1.5 ÃÂ²2 and Nav1.5 ÃÂ²4 subunits. Considering the interaction between Nav1.5 and its ÃÂ² subunits may provide novel targets for antiarrhythmic drug therapy.

222 AUGMENTATION OF CREATINE KINASE IN VITRO PROTECTS AGAINST SIMULATED ISCHAEMIA REPERFUSION INJURY

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10.1136/heartjnl-2017-311726.220

Creatine kinase (CK) catalyses the interchange of high energy phosphates to buffer ATP levels and maintains cellular energy homeostasis. The heart expresses three isoforms: sarcomeric mitochondrial CK (CKMT2), and the cytoplasmic CKM and CKB isoforms which form homo (MM/BB)- and hetero (MB)-dimers. Impaired CK activity is associated with heart failure and increases susceptibility to ischaemia/reperfusion injury.

We hypothesised that augmentation of CK isoenzymes *in vitro* would improve cell viability following exposure to hypoxia/reoxygenation. For this purpose we created CK overexpression systems by cloning the open reading frame of the different CK isoform sequences into pcDNA3.1 expression vector and stably selected and characterised overexpressing HEK293 cell lines.

The generated cell lines displayed increased CK activity in addition to individual CK isoenzyme activities. CKMT2, CKM and CKB cells had elevated total CK activity (p<0.001; p<0.001; p<0.01 One-way ANOVA, Dunnetts post-test vs HEK293). Furthermore immunocytochemistry showed that CKMT2 co-localises with mitochondrial marker COXIV in the intermembrane space following transient transfection in HL1 atrial cell line.

Both stable and untransfected HEK293 cells were exposed to simulated ischaemia/reperfusion by incubating at 1% O_2 for 18 hour, followed by re-oxygenation at 95% O_2 for 2 hour. The positive control rapamycin was supplemented into the cell media 4 hours prior to hypoxia. Viability analysis by propidium iodide detection using a CyAN flow cytometer at 488 nm, showed increased cell survival by 33% in CKMT2, 47% in CKM and 58% in CKB cells when compared to untransfected HEK293 controls (in all cases p<0.05, One-Way ANOVA Dunnetts post-test vs HEK293).

To determine whether protection was due to changes in antioxidant capacity, cells were loaded with the reactive oxygen species indicator dye, $DCFH_2$-DA, and exposed to H_2O_2-induced oxidative stress. Overexpression of CK isoenzymes failed to attenuate fluorescence from oxidised dye in contrast to the known antioxidant, Trolox. Transient expression of CK constructs in the HL1 cell line was used to test the effects of anthracycline exposure on cell viability (48 hour doxorubicin). Pre-treatment with Trolox increased cell survival by 12.4% (79.4%±2.0 vs. 67%±1.5 in empty-vector control cells; p<0.01) whereas overexpression of CK isoenzymes did not alter cell death rates.

In conclusion, overexpression of any one (of three) cardiac creatine kinase isoenzymes protects against ischemia/reperfusion *in vitro*. This most likely reflects enhanced energy reserve due to elevated CK activity, since response to oxidative challenge was unaltered. Further mechanistic studies and *in vivo* confirmation of these findings are merited.

223 INVESTIGATING THE ROLE OF AEROBIC GLYCOLYSIS IN ARTERIAL CALCIFICATION

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10.1136/heartjnl-2017-311726.221

Objective The process of arterial calcification shares many similarities to skeletal mineralisation, and involves the deposition of hydroxyapatite in the arteries. However, the cellular mechanisms responsible have yet to be fully elucidated. Accumulating evidence suggests that aerobic glycolysis (the Warburg effect), plays a critical role in meeting the demand for energy and biosynthetic precursors during proliferation and differentiation in numerous cell types. Therefore we addressed the hypothesis that vascular smooth muscle cell (VSMC) calcification requires aerobic glycolysis to produce energy and the necessary biosynthetic precursors.

Methods Calcification of murine aortic VSMCs was induced by 3 mM Pi for 7 days. Calcium deposition was determined using alizarin red staining and a modified o-cresolphthalein method. VSMCs were cultured with the fluorescent glucose analogue 2-(N-(7-Nitrobenz-2-oxa-1,3-diazol-4-yl)Amino)−2-Deoxyglucose (2-NBDG) to determine changes in glucose uptake. Gene expression was analysed by qRT-PCR.

Results Calcium deposition was significantly increased in VSMCs cultured in 3 mM Pi versus control conditions (124%, p<0.001). Calcified VSMCs also showed increased mRNA expression of Runx2, Phospho1, Ocn and PiT-1 (p<0.001), recognised osteogenic markers of arterial calcification.

Furthermore 3 mM Pi treatment increased glucose uptake (98%, $p<0.05$) and Glut-1 mRNA expression (1.47 fold, $p<0.001$). Glycolysis converts glucose to pyruvate which is subsequently converted to either (i) acetyl-CoA by the pyruvate dehydrogenase complex (PDH) or (ii) lactate by lactate dehydrogenase (LDH). Notably, decreased VSMC calcification was observed in cells treated with sodium dicholoroacetate, an inducer of PDH activity (1 mM; 40%; $p<0.01$) and citric acid, synthesised in the mitochondria from acetyl CoA (1 mM; 72%, $p<0.001$). Treatment with the LDH inhibitor sodium oxamate (20 mM) or sodium lactate (50 mM) to induce pyruvate production also inhibited VSMC calcification (68% and 53% respectively, $p<0.05$). Activation of the Wnt pathway – an established regulator of Warburg metabolism – using the selective GSK3 inhibitor CHIR99021 (1 nM) significantly increased VSMC calcification (417%, $p<0.001$). However, co-treatment with sodium oxamate (20 mM) significantly blunted the pro-calcification effect of CHIR99021 (69%, $p<0.01$).

Conclusion Together these data suggest that arterial calcification requires glucose metabolism through a mechanism involving Wnt signalling. Interruption of the glycolysis pathway may therefore represent a novel therapeutic target for clinical intervention.

224 VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR INHIBITION INDUCES VASCULAR DYSFUNCTION VIA REDOX-SENSITIVE PROCESSES

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10.1136/heartjnl-2017-311726.222

Vascular endothelial growth factor (VEGF)/VEGF receptor (VEGFR) inhibitors, used as anti-angiogenic drugs to treat cancer, induce severe hypertension. Molecular mechanisms whereby VEGF inhibitors cause hypertension are unclear, but nitric oxide (NO) and oxidative stress may be involved. We questioned whether reactive oxygen species (ROS), important regulators of vascular function in hypertension, also play a role in VEGF inhibitor-induced vascular dysfunction. Human microvascular endothelial cells (HMECs) were stimulated with vatalanib (VEGFR inhibitor) and gefitinib (EGFR inhibitor). Normotensive male SV-129 mice (8 weeks old) were treated with Vatalanib (100 mg/Kg/day) or Gefitinib (100 mg/Kg/day). Vascular reactivity was performed mesenteric arteries using wire myograph and blood pressure was measured by tail-cuff method. Phosphorylation of eNOS was assessed by immunoblotting. ROS were measured by amplex red, lucigenin and nitrotyrosine elisa. TBARS levels were measured by lipid peroxidation assay kit and catalase activity by amplex red. Nox and antioxidant enzymes mRNA was analysed by qPCR. No changes in blood pressure were observed in animals treated with vatalanib on this dose. However acetylcholine (ACh)-induced vasodilatation was impaired in those mice and phosphorylation of eNOS activation site (Ser1177) was decreased, while no changes were observed after exposure of HMECs to gefitinib. Hydrogen peroxide (H_2O_2) levels were reduced in HMECs stimulated with vatalanib and in aorta and heart from vatalanib-treated mice. This effect was followed by an increase in catalase activity and a decrease in Nox 4 mRNA expression while Nox5 mRNA levels were increase by vatalanib. VEGF inhibition also increased peroxynitrite ($ONOO^-$) levels in aorta and kidney and increased plasma TBARS levels. In kidney vatalanib increased H_2O_2 and O_2^- production which was followed by a decrease in catalase activity and Nrf2 nuclear translocation. Finally mRNA levels of antioxidant enzymes in HMECs, kidney and heart were decreased after exposure to vatalanib. Gefitinib only increased catalase activity and $ONOO^-$ levels in heart as well as decreased Nrf2 nuclear translocation in kidney from mice. In conclusion, our data identify novel mechanisms whereby VEGFR inhibition modulates NO signalling, antioxidant defences and ROS production in tissues and endothelial cells. These molecular processes may contribute to reduced vasorelaxation and may play a role on VEGFRI-induced hypertension.

225 THE ROLE OF THE DNA DAMAGE RESPONSE IN VASCULAR CALCIFICATION

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10.1136/heartjnl-2017-311726.223

Introduction Vascular calcification is a hallmark of vascular ageing, and associated with vascular smooth muscle cell (VSMC) death, phenotype modulation and maladaptation. However, it remains unclear how the initial stress signals link to these downstream cellular events. Emerging evidence and our *in vitro* data suggest that stress may drive vascular calcification through elevated levels of DNA damage, a key factor driving cellular ageing.

Methods and Results We investigated the effects of different DNA damage signalling pathway inhibitors (ATM, ATM/ATR and PARP1) on the progression of vascular calcification. Using comet assays and western blot, we found elevated levels of DNA damage in calcified VSMCs and that inducing DNA damage accelerated rates of calcification. Chemical inhibition or siRNA knockdown of ATM, ATM/ATR or PARP signalling reduced or delayed calcification and prevented cells undergoing calcification-associated phenotype changes including osteo/chondrogenic differentiation. Prevention was associated with down-regulation of senescence and inflammatory markers suggesting the senescence associated secretory phenotype (SASP) acts to potentiate VSMC calcification.

Conclusion Taken together, these *in vitro* data suggest DNA damage signalling is involved in the pathological regulation of calcification. Therefore, interventions that reduce DNA damage, promote DNA damage repair, or modulate DNA damage